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New Drugs

Trastuzumab, a Recombinant DNA–Derived Humanized Monoclonal Antibody, a Novel Agent for the Treatment of Metastatic Breast Cancer

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ABSTRACT

Amplification of the human epidermal growth factor receptor 2 protein (HER2) in primary breast carcinomas has been shown to correlate with poor clinical prognosis for certain patients. Trastuzumab (Herceptin®, Genentech, Inc., South San Francisco, California) is a highly purified recombinant DNA–derived humanized monoclonal immunoglobulin G1 kappa antibody that binds with high affinity and specificity to the extracellular domain of the HER2 receptor. In vitro and in vivo preclinical studies have shown that administration of trastuzumab alone or in combination with paclitaxel or carboplatin significantly inhibits the growth of breast tumor–derived cell lines that overexpress the *HER2* gene product. At therapeutic doses in breast cancer patients, the mean half-life of trastuzumab is 5.8 days. Trastuzumab serum concentrations reach steady state with mean trough and peak concentrations of 79 μg/mL and 123 μg/mL, respectively. In a 222-patient, single-arm clinical study, treatment with a loading dose of trastuzumab 4 mg/kg administered IV followed by weekly IV doses of 2 mg/kg produced an overall response rate of 14% (2% complete remission and 12% partial remission). The beneficial effects were greatest in patients with the greatest degree (3+) of HER2 protein overexpression. In another clinical study, 469 women with metastatic breast carcinoma were randomized to a paclitaxel or anthracycline-plus-cyclophosphamide regimen with or without trastuzumab. The overall response rate was significantly greater in the trastuzumab-plus-chemotherapy group than in the chemotherapy-alone cohort. The magnitude of observed effects was greatest with pacli-

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taxel plus trastuzumab. The most common adverse effects attributed to trastuzumab in clinical studies were fever and chills, pain, asthenia, nausea, vomiting, increased cough, diarrhea, headache, dyspnea, infection, rhinitis, and insomnia. Trastuzumab in combination with chemotherapy can lead to cardiotoxicity, leukopenia, anemia, diarrhea, abdominal pain, and infection. Trastuzumab has been approved by the US Food and Drug Administration as a single agent for the treatment of patients who have metastatic breast cancer involving overexpression of the HER2 protein and who have received 1 or more chemotherapy regimens; in combination with paclitaxel, it has been approved for the treatment of such patients who have not received chemotherapy. ***Key words:*** trastuzumab, HER2 overexpression, metastatic breast cancer, rhuMabHER2.

INTRODUCTION

Based on data in the Surveillance, Epidemiology, and End Results database of the National Cancer Institute, the age-adjusted incidence of invasive breast cancer in white and black women for the years 1990 and 1995 was 114.5 and 100.5 cases per 100,000 persons, respectively; the mortality rate was 26 and 31.5 cases per 100,000 persons, respectively.[1] It was estimated that breast cancer (excluding breast carcinoma in situ) would be newly diagnosed in 178,700 women and be the reported cause of death in 43,500 women in 1998.[2] In 1994, stage IV or metastatic breast cancer constituted 3.6% and 6.2% of all breast cancer types among white and black women, respectively.[3] It has been estimated that over 80,000 women per year will develop metastatic or refractory breast cancer, with rates of metastatic disease having remained constant in white women between 1973 and 1993.[4,5]

Growth factors and their receptors play pivotal roles in the regulation of cell growth and differentiation.[6] Malignancy arises from a stepwise progression of genetic events that often includes unregulated expression of growth factor receptors or elements of their signaling pathways.[6,7] Overexpression, or amplification, of the human epidermal growth factor receptor (EGFR) 2 protein (HER2), which is correlated with poor clinical outcome in patients with breast cancer, is believed to result from gene amplification.[8] This protein is located on the cell's surface, where it interacts with growth factors. When the HER2 protein is overexpressed, the cells divide, grow, and multiply at a faster rate than normal, contributing to the development of cancer. When both node-negative and node-positive breast cancers were reviewed, a significant difference in 5-year survival was found between primary breast cancers that overexpressed HER2 compared with those that did not.[9] Studies in breast cancer patients have shown that 25% to 30% of breast cancers overexpress the *HER2* gene.[10]

The *HER2* gene (also known as *neu* and *c-erbB-2*) encodes a 185-kd transmembrane/kinase receptor, designated p185HER2, that has partial homology with the other members of the EGFR family.[11–13] Antibodies directed against HER2 can inhibit the growth of tumor xenografts and transformed cells that express high concentrations of this receptor.[14,15] The murine monoclonal antibody mumAB4D5, directed against human p185HER2, has been shown to specifically inhibit proliferation of human tumor cells overexpressing this receptor.[16] Amplification or overexpression of HER2 is associated with multiple human malignancies, especially breast can-

cer,[17] and with more rapid cancer progression and shortened survival.[10] A similar but less frequent amplification of the *HER2/c-erbB-2* gene has been described in gastric adenocarcinoma.[18]

DESCRIPTION OF TRASTUZUMAB

A recombinant humanized anti-HER2 antibody, trastuzumab (Herceptin®, Genentech, Inc., South San Francisco, California) was constructed and developed to determine whether its use, either alone or in combination with chemotherapeutic agents, could reduce the progression of malignancy in women with advanced breast cancer. Assays were performed both in vitro and in animal models to find whether the recombinant human monoclonal antibody inhibited the proliferation of tumor cells overexpressing HER2. The agent's safety was also a critical concern of these studies.

Trastuzumab is a highly purified recombinant DNA–derived humanized monoclonal immunoglobulin G1 kappa antibody that in a cell-based assay (kd = 5 nmol), binds selectively and with high affinity to the extracellular domain of the human EGFR2 protein, HER2.[10,17] It is produced by mammalian cell culture using Chinese hamster ovary suspension culture in a nutrient medium containing the antibiotic gentamicin. The antibiotic is not detectable in the final product. The approximate weight of the antibody is 148 kd.

Trastuzumab binds with high affinity and specificity to the extracellular domain of the HER2 receptor that is overexpressed in some breast cancer cells. The antibody contains human framework regions, with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.[15]

PRECLINICAL STUDIES

In 1 study,[19] SKBR3 human breast tumor cells overexpressing the *HER2/c-erbB-2* gene or A431 human squamous carcinoma cells overexpressing the *EGFR* gene were grown in flasks. The cells were detached from the flasks by treatment with 25 mmol/L ethylenediamine–tetraacetic acid/0.15 mol/L sodium chloride, collected by low-speed centrifugation, and suspended at 1×10^6 cells/mL in phosphate buffered saline/1% fetal bovine serum. Each cell line (1 mL) was incubated with 10 μg of either anti-HER2/c-erbB-2 monoclonal antibody (4D5) or a control antibody recognizing the hepatitis B surface antigen. The monoclonal antibody 4D5 was bound to the surface cells of the human tumor cell line expressing p185HER2, as measured by fluorescence-activated cell sorting. There was a 160-fold increase in cellular fluorescence compared with a control monoclonal antibody when 4D5 was added to SKBR3 breast adenocarcinoma cells. This cell line contains an amplified *HER2/c-erbB-2* gene and expresses high concentrations of p185HER2.[19,20] In contrast, the squamous carcinoma cell line A431, which expresses about 2×10^6 EGFR per cell[21] but only low concentrations of p185HER2, exhibited only a twofold increase in fluorescence with 4D5 compared with a control monoclonal antibody.

Most anti-HER2/c-erbB-2 monoclonal antibodies that recognize the extracellular domain inhibited the growth of SKBR3 cells in this study.[19] Maximum inhibition was obtained with monoclonal antibody 4D5, which inhibited cellular proliferation by 56%. The control antibodies had no significant effect on cell growth.

In another study,[16] the humanized monoclonal antibody 4D5-8 was found to

bind to p185HER2 with high affinity (250 times control) and to prevent the proliferation of the human mammary adenocarcinoma cell line SKBR3. The monoclonal antibody also promoted antibody-dependent cellular toxicity against SKBR3 tumor cells in the presence of human effector cells but was not effective in directing the killing of normal (WI-38) cells, which express p185HER2 at much lower concentrations. This finding predicted effective treatment of human cancers that overexpress p185HER2 with human antibody 4D5-8.

The results of these 2 studies indicate that trastuzumab has a higher affinity for p185HER2 (kd = 0.1 nmol) than does the murine Mab 4D5 and has a cytostatic growth-inhibitory effect against breast cancer cells overexpressing HER2. An additional in vitro study[22] suggests that the growth of those breast cancer cell lines having the highest basal concentration of p185HER2 is most inhibited by the anti-p185HER2 antibody.

In another in vitro study,[23] a significant synergistic suppression of cell proliferation was observed between 4D5 antibody and cisplatin in SKBR3 breast carcinoma cells overexpressing the *HER2/neu* gene ($P < 0.001$). Such synergistic activity was also noted when 4D5 was added to SKBR3 cells in combination with carboplatin ($P < 0.001$). To confirm the relative receptor-dependent specificity of this phenomenon, C13pRV-CON cells that did not overexpress the *HER2/neu* proto-oncogene were treated with identical antibody/drug combinations, and no apparent synergistic decrease in cell growth was observed. The synergistic effect of the combination of 4D5 and cisplatin was confirmed in vivo in tumor-bearing mice, where significant and marked inhibition of tumor growth was observed that exceeded the effect of either agent alone ($P < 0.005$).

In a study of tumor growth in athymic nude mice,[24] treatment with rhuMabHER2 0.1 to 30 mg/kg intraperitoneally twice weekly plus paclitaxel 5 to 10 mg/kg IV on days 1 and 4 significantly enhanced inhibition of tumor growth (93%, $P = 0.006$) compared with either agent administered alone. In addition, tumor growth inhibition at 5 weeks was significantly superior in the group treated with the monoclonal antibody plus paclitaxel compared with paclitaxel alone ($P = 0.016$) but not rhuMabHER2 alone ($P = 0.4$). Therapy combining the antibody with doxorubicin inhibited growth by 70% versus control-treated mice ($P = 0.04$) but was not statistically superior to doxorubicin alone ($P = 0.16$) or the antibody alone ($P = 0.59$).

PHARMACOKINETICS

The pharmacokinetic properties of trastuzumab have been studied in patients with metastatic breast cancer.[25] Short-duration IV infusions of 10 to 500 mg once weekly showed dose-response kinetics. That is, mean half-life increased and clearance decreased with increasing doses. The half-life averaged 1.7 and 12 days at the 10- and 500-mg doses, respectively. The volume of distribution was approximately equal to that of serum volume (44 mL/kg). At the highest weekly dose (500 mg) studied, mean peak serum concentrations were 377 μg/mL.

In studies of trastuzumab using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, the mean half-life was 5.8 days (range, 1 to 32 days).[26] Between weeks 16 and 32, trastuzumab serum concentrations reached

steady-state with mean trough and peak concentrations of approximately 79 and 123 μg/mL, respectively.

Detectable concentrations of the circulating extracellular domain of the HER2 receptor (shed antigen) were found in the serum of some patients with tumors overexpressing HER2. Determination of shed antigen in baseline serum samples revealed that 64% of patients had detectable shed antigen at concentrations ranging as high as 1.88 μg/mL. Patients with higher baseline concentrations of shed antigen were more likely to have lower serum trough concentrations of trastuzumab. However, with weekly dosing, most patients with elevated concentrations of shed antigen achieved target serum concentrations of trastuzumab by week 6.[25]

CLINICAL EXPERIENCE

A study was conducted in 46 patients with metastatic breast cancer that overexpressed HER2.[27] Patients received a loading dose of 250 mg trastuzumab IV over a 90-minute period (day 0), followed by one 100-mg dose for 10 weeks beginning on day 7. At the conclusion of the treatment period, patients with stable disease or a minor, partial, or complete response were entered into a maintenance phase consisting of weekly trastuzumab administration until disease progression.

All patients had measurable disease, had a Karnovsky performance status of ≥60% (required occasional help carrying out normal activities), and retained hematologic, hepatic, renal, and pulmonary function. Chemotherapy or additive hormone therapy was not permitted within 3 weeks before study entry (6 weeks for mitomycin or nitrosoureas). Tumor expression of HER2 was determined by immunohistochemical analysis.[8,10]

Tumor response was ascertained at the completion of the initial 11-week treatment period. Complete response was defined as the disappearance of all radiographically or visually apparent tumors; partial response as a ≥50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions; minimal response as a ≥25% and <50% reduction in the diameters of all measurable lesions; stable disease as no change ≥25% in the size of any measurable lesion; and progressive disease as a >25% increase in any measurable lesion or the appearance of any new lesion. To be considered responders, patients had to have achieved at least stability of bone lesions. Time to tumor progression was calculated from the beginning of therapy to progression.

Forty-three patients were assessable for treatment response on day 77. Three patients were not assessable for response: 1 had a bacteremic infection of an IV catheter that required prolonged antibiotic administration, precluding treatment with trastuzumab; 1 patient discontinued treatment for personal reasons; and 1 patient died of congestive heart failure associated with prior doxorubicin treatment. The overall response rate to trastuzumab (complete plus partial responses) was 11.6% (95% confidence interval, 4.36 to 25.9) in the 43 patients. Two patients had a minimal response, and 14 patients had stable disease at day 77. These patients entered a maintenance phase consisting of weekly antibody administration until progression of disease. The median time to progression for patients with either minimal or stable disease was 5.1 months.

An additional patient had a >50% reduction in the size of the metastatic le-

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